

Effects of Ultraviolet-B Radiation on
Plants during Mild Water Stress. 3
Effects on Photosynthetic Recovery and
Growth in Soybean

Maryland Univ., College Park

Prepared for

Corvallis Environmental Research Lab., OR

1984

TECHNICAL REPORT DATA (Please read Instructions on the reverse before completing)		
1. REPORT NO. EPA-600/J-84-073	2.	PB84-233436
4. TITLE AND SUBTITLE Effects of ultraviolet-B radiation on plants during mild water stress. III. Effects on photosynthetic recovery and growth in soybean (Journal Version)	5. REPORT DATE 1984	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) Alan H. Teramura, Mark C. Perry, John Lydón, Marla S. McIntosh and Edwin G. Summers	8. PERFORMING ORGANIZATION REPORT NO.	
9. PERFORMING ORGANIZATION NAME AND ADDRESS University of Maryland, College Park, MD 20742	10. PROGRAM ELEMENT NO.	11. CONTRACT/GRANT NO. CR 808 035-020
12. SPONSORING AGENCY NAME AND ADDRESS Environmental Research Laboratory Office of Research and Development - U.S. EPA 200 SW 35th Street Corvallis, Oregon 97333	13. TYPE OF REPORT AND PERIOD COVERED Journal Article	14. SPONSORING AGENCY CODE EPA/600/02
15. SUPPLEMENTARY NOTES Published in: <i>Physiol. Plant.</i> 60: 484-492. Copenhagen 1984		
16. ABSTRACT Soybean was grown from seed in a greenhouse under ultraviolet-B (UV-B, 280-320 nm) radiation supplied by filtered FS-40 sunlamps. On a weighted, total daily dose basis these plants received either 0 (control) or 2875 effective $J\ m^{-2}\ day^{-1}$ UV-B _{BE} . When weighted with the generalized plant action spectrum, this simulated the solar ultraviolet-B irradiance expected to occur at College Park, Maryland, USA (39 degrees N) in the event the global stratospheric ozone column is reduced by 23%. The effects of ultraviolet radiation on the photosynthetic recovery from water stress were measured with an infrared gas analyzer. These effects were examined in plants which were either well-watered or previously preconditioned to water stress, during two distinct phenological stages of development. During the early stages of soybean growth, enhanced levels of UV-B reduced net photosynthesis by 25%, and water stress also reduced photosynthesis to nearly the same extent (by 20%). The combination of these two stresses resulted in smaller biomass than that produced by plants exposed to either stress independently. Photosynthesis in older, larger plants was much more sensitive to water stress and was reduced by as much as 50-60% in non-preconditioned plants. Although non-irradiated, non-preconditioned (control) plants recovered to only within 60% of their prestressed value, preconditioned plants recovered to within 70-80% during the 3 day recovery period.		
17. KEY WORDS AND DOCUMENT ANALYSIS		
a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
18. DISTRIBUTION STATEMENT Release to public	19. SECURITY CLASS (This Report) Unclassified	21. NO. OF PAGES 11
	20. SECURITY CLASS (This page) Unclassified	22. PRICE

Effects of ultraviolet-B radiation on plants during mild water stress. III. Effects on photosynthetic recovery and growth in soybean

Alan H. Teramura, Mark C. Perry, John Lydon, Maria S. McIntosh and Edwin G. Summers

Teramura, A. H., Perry, M. C., Lydon, J., McIntosh, M. S. and Summers, E. G. 1984. Effects of ultraviolet-B radiation on plants during mild water stress. III. Effects on photosynthetic recovery and growth in soybean - *Physiol. Plant.* 60: 484-492.

Soybean [*Glycine max* (L.) Merr. cv. Essex] was grown from seed in a greenhouse under ultraviolet-B (UV-B, 280-320 nm) radiation supplied by filtered FS-40 sunlamps. On a weighted, total daily dose basis these plants received either 0 (control) or 2875 effective $\text{J m}^{-2} \text{day}^{-1}$ UV-B_{DE}. When weighted with the generalized plant action spectrum (Caldwell 1971), this simulated the solar ultraviolet-B irradiance expected to occur at College Park, Maryland, USA (39°N) in the event the global stratospheric ozone column is reduced by 23%. The effects of ultraviolet radiation on the photosynthetic recovery from water stress were measured with an infrared gas analyzer. These effects were examined in plants which were either well-watered or previously preconditioned to water stress, during two distinct phenological stages of development. During the early stages of soybean growth, enhanced levels of UV-B reduced net photosynthesis by 25%, and water stress also reduced photosynthesis to nearly the same extent (by 20%). The combination of these two stresses resulted in smaller biomass than that produced by plants exposed to either stress independently. Photosynthesis in older, larger plants was much more sensitive to water stress and was reduced by as much as 50-60% in non-preconditioned plants. Although non-irradiated, non-preconditioned (control) plants recovered to only within 60% of their prestressed value, preconditioned plants recovered to within 70-80% during the 3 day recovery period. Both water stress and UV-B radiation affected non-stomatal conductance, while stomatal conductance was primarily affected by water stress.

Additional key words - *Glycine max*, ozone depletion, interactions.

A. H. Teramura (reprint requests), M. C. Perry, J. Lydon, Dept of Botany, Univ. of Maryland, College Park, MD 20742, USA; M. S. McIntosh, Dept of Agronomy, Univ. of Maryland; E. G. Summers, Dept. of Horticulture, Univ. of Maryland.

Introduction

The deleterious effects of non-optimal growth conditions or plant stress on physiological processes have been the subject of many studies. Two stress factors that have received attention are enhanced ultraviolet-B (280-320 nm) radiation and drought. Inadequate soil moisture leading to plant water stress is a common phenomenon in many parts of the world. Considering world vegetation types, plant productivity is more closely related to available water than to any other environmental factor (Salisbury and Ross 1978). Water deficits exist in many plants both diurnally and during dry periods between irrigation or rainfall cycles. Water stress affects plant growth pri-

marily through the alteration of most physiological processes and the reduction in turgor necessary for cell expansion (Hsiao 1973).

In actual field conditions it is common for a plant to encounter more than one environmental stress simultaneously. Although the net effect of two or more concomitant stresses on a given physiological or growth characteristic can either be antagonistic or additive, it is possible that multiple stresses have synergistic negative effects. An extensive body of literature has accumulated which documents the effects of water deficits on plant growth and development (for review see Hsiao 1973). Within the past decade, increasing concern over the pos-

Received August 10, 1983; revised 4 December, 1983

sibility of a partial stratospheric ozone depletion (National Academy of Sciences 1982) has also generated considerable interest and increased our understanding of the effects of enhanced UV-B radiation on plants (for reviews see Caldwell 1981, Teramura 1983). However, to date, only a few studies (Teramura et al. 1983, Tevini et al. 1983b) have examined the combined effects of both stresses supplied simultaneously. Knowledge of the effects of such combined stresses could greatly increase our understanding of the potential impacts of partial stratospheric ozone depletion under actual field conditions, since plants might be expected to simultaneously encounter both stresses in nature.

The purpose of this study was threefold: 1) to investigate the effects of simultaneous exposure of soybeans to enhanced ultraviolet-B radiation and water stress on growth and photosynthetic recovery after water stress was alleviated; 2) to determine whether different phenological stages of development respond differently; and 3) to determine if preconditioning plants to drought stress facilitated more rapid recovery from subsequent water stress.

Abbreviations – PPFD, photosynthetic photon flux density, 400–700 nm; SLW, specific leaf weight; Trt, treatment; UV-B, ultraviolet radiation between 280–320 nm; UV-B_{RE}, biologically effective UV-B radiation weighted with generalized plant response function (Caldwell 1971); UV-B_{DNA}, biologically effective UV-B radiation weighted with DNA action spectrum (Setlow 1974); –UV–W, preconditioned to water stress and no UV-B radiation; +UV–W, preconditioned to water stress and simultaneously exposed to UV-B radiation; +UV+W, not preconditioned to water stress and simultaneously exposed to UV-B radiation; –UV+W, not preconditioned to water stress and no UV-B radiation (control); ψ , leaf water potential.

Materials and methods

Plant growth conditions

Soybean seeds [*Glycine max* (L.) Merr. cv. Essex] were planted, 4 seeds per pot, in plastic 1 l pots in a 1:1 (v:v) sterile medium of perlite and potting soil at the University of Maryland greenhouse. Plants were thinned to 1 per pot 6 days after emergence and inoculated with *Rhizobium japonicum* (strain 110) at the appearance of the first unifoliate leaf. Beginning with the appearance of the first trifoliate leaf, plants were fertilized weekly with full strength 20–20–20 fertilizer (N, P, K), every 4th day with 100 ml of half-strength modified Hoagland solution (Hoagland and Arnon 1950), and watered daily to field capacity with an automatic watering system. Average air temperature in the greenhouse was 25°C and relative humidity varied daily from 50 to 90%. Daily photosynthetic photon flux density (PPFD, 400–700 nm) recorded with a LiCor LI-550 Printing Integrator averaged $1.8 \times 10^7 \mu\text{mol m}^{-2} \text{s}^{-1}$ and mean daily maximum PPFD at plant height was near $1400 \mu\text{mol m}^{-2} \text{s}^{-1}$ as measured with a LiCor quantum sensor. The natural photoperiod was approximately 14 h.

The experimental design was a completely randomized split plot with the following 4 treatments:

- Trt (1) not preconditioned to water stress and simultaneously exposed to UV-B radiation (+UV+W)
- Trt (2) not preconditioned to water stress and no UV-B radiation (–UV+W) (control)
- Trt (3) preconditioned to water stress and simultaneously exposed to UV-B radiation (+UV–W)
- Trt (4) preconditioned to water stress and no UV-B radiation (–UV–W)

Each of the above treatments was repeated at 2 different phenological stages of development (one vegetative and one reproductive) making a total of 8 treatments. Plants ($n = 240$) were randomized under 5 replications of 2 radiation levels (UV-B radiation and control) and the 2 water stress treatments and 2 growth stages were randomized within these. There were 6 plants in each treatment per replication. The irradiation compartments were separated by a 0.13 mm Mylar Type S plastic barrier which absorbs essentially all radiation of wavelengths shorter than 315 nm.

UV-B radiation was supplied by filtered Westinghouse FS-40 sunlamps suspended above the plants. Lamps were held in racks which were attached to pulleys, which facilitated the maintenance of 0.25 m between the lamp and plants as they grew. All lamps were fitted with either presolarized 0.08 mm cellulose acetate (UV-B transmitting) or 0.13 mm Mylar Type S (control) filters. The spectral irradiance 0.25 m beneath the lamps is shown in Fig. 1. Cellulose acetate filters were replaced every 70 h while Mylar filters were changed at 120 h

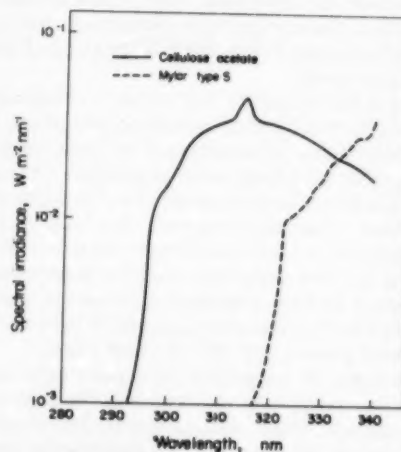


Fig. 1. The spectral distribution of UV-B radiation 0.25 m beneath filtered FS-40 sunlamps.

intervals. Lamps were preburnt for at least 100 h prior to use to ensure uniformity of radiation (Teramura et al. 1980); and plants were re-randomized under each radiation treatment at 5 day intervals. Spectral irradiance of the lamps was determined with an Optronics Laboratories Inc. Model 742 Spectroradiometer interfaced with a Hewlett Packard 85 printing calculator. The spectral irradiance was weighted using two different biological action spectra. Plants were irradiated for 5 h on either side of solar noon, providing a daily dose of 2875 effective $\text{J m}^{-2} \text{ day}^{-1}$ UV-B_{BE} when weighted with a generalized plant action spectrum (Caldwell 1971) and 341 effective $\text{J m}^{-2} \text{ day}^{-1}$ UV-B_{DNA} when a DNA weighting function (Setlow 1974) was used. On a daily dose basis when weighted with the generalized plant action spectrum (Caldwell 1971), this is approximately equivalent to that radiation received should a 23% ozone reduction occur with clear sky conditions in College Park, Maryland, USA (39°N latitude) during the summer solstice. UV-B irradiation began immediately after germination and continued throughout the water stress and recovery periods.

Water stress was imposed by withholding water from designated pots. At the point of incipient wilting, total leaf water potential (ψ) was measured on the center leaflet of a trifoliate with a PMS Company Model 1000 Pressure Chamber. At this point ψ was approximately -2.0 MPa. Plants were stressed to this point and then rewatered for each of 3 drought cycles.

The main experimental plots consisted of 120 plants for each phenological stage. Border rows were used around each main plot to minimize potentially unequal PPFDs within the greenhouse. The phenological stages studied were identified using the terminology of Fehr and Caviness (1977) as: V-3, during the initiation of the 3rd trifoliate leaf or approximately 16 days after emergence; and R-2, the stage where a flower is found at a node immediately below the uppermost node with a completely unrolled leaf. These stages were chosen such that stress cycles did not progress into the flowering or pod filling stages, respectively.

At the initiation of the 3rd trifoliate in vegetative plants and the 11th trifoliate in reproductive plants, half of the plants were preconditioned to water stress by allowing them to dry to the initial point of wilt. They then were watered daily to field capacity for 3 days and dried again. After 3 days recovery, both these and the non-preconditioned plants (controls) were dried to ψ of -2.0 MPa (Fig. 2). Time required to reach this point varied as a function of ambient temperature, insolation, and size of the plant as V-3 stage plants required 5 days and R-2 stage plants reached -2.0 MPa in about 2 days.

Dry weights of component plant parts were determined immediately after recovery from the final water stress treatment. Plants were 36- and 60-days old at the time of harvest for vegetative and reproductive plants, respectively. Ten plants from each treatment were harvested and separated into leaves, stems, petioles and

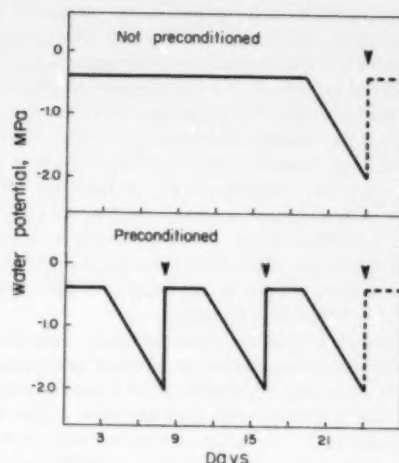


Fig. 2. Preconditioning to water stress was achieved by withholding water and allowing the plants to dry. Arrows indicate when plants were rewatered to field capacity. The photosynthetic recovery from water stress was monitored daily after the last drying cycle (dashed lines).

roots. Leaf areas were obtained using a LiCor Model LI-3100 Area Meter. Tissue samples were dried for 48 h at 60°C and weights were determined.

Gas exchange and pigment analyses

Gas exchange measurements were conducted on the center leaflet on either the 3rd trifoliate (for the V-3 stage) or the 11th trifoliate (R-2 stage) before the last water stress cycle commenced and 20, 44 and 68 h after the final rewatering. Net photosynthesis and dark respiration were measured on single, attached leaves using an Anarad 600R dual beam, non-dispersive infrared gas analyzer operating in an open system. This instrument measured the fluxes of CO_2 and H_2O vapor simultaneously, enabling calculations to be made for net photosynthesis, transpiration and the diffusion conductance to CO_2 and H_2O vapor (Jarvis 1971). The average ambient CO_2 and H_2O vapor concentrations during measurement were $349 \mu\text{l l}^{-1}$ and 16.7 mg l^{-1} , respectively. Dark respiration was determined by placing a light-tight cloth bag over the chamber and measuring the efflux of CO_2 . Light during measurements of photosynthesis was supplied by Westinghouse 300 W low temperature, wide flood PAR lamps (Model 300 PAR 56/2WFL), filtered through 0.10 m of continuously flowing water. Photosynthetic photon flux density incident on the leaf during net photosynthesis measurement was $1200 \mu\text{mol m}^{-2} \text{ s}^{-1}$. Stomatal conductance to H_2O vapor was measured with a LiCor Model LI-65 Autoporometer. Non-stomatal conductance was calculated as a residual term, using 1.56 as the factor relating H_2O and CO_2 diffusivities.

Chlorophyll and flavonoid concentrations were deter-

mined on the same trifoliolate used for the gas exchange measurements and expressed on a leaf area basis. Chlorophyll concentration was determined according to Arnon (1949) and flavonoid determinations according to Caldwell (1968). The absorbance of acidified (1% v:v) methanolic extracts was measured by scanning between 250–360 nm on a Perkin-Elmer Model 124 Double Beam Grating Spectrophotometer and Perkin-Elmer Model 165 recorder. The absorbance at 340, 290 and 270 nm peaks was used for comparison among treatments.

Statistical analysis was done using the Statistical Analysis System (SAS), incorporating the General Linear Model Procedure (Goodnight 1979). To test for significant differences, a 2-way analysis of variance (ANOVA) was performed on growth and gas exchange characteristics (Sokal and Rohlf 1981). Means were separated by a Duncan's Multiple Range Test at the 5% level unless otherwise stated.

Results

Photosynthetic recovery

Non-preconditioned, vegetative plants which were irradiated with UV-B (+UV+W; Trt 1) showed larger reductions in net photosynthesis than non-irradiated plants (–UV+W; Trt 2) before the onset of water stress (day = 0), as well as throughout the 3 day recovery period (Fig. 3A). None of the non-preconditioned plants fully recovered photosynthetically from the stress cycle. Reductions in net photosynthesis resulting from both UV and water stress were not the result of lower non-stomatal conductance (Tab. 1). Stomatal conductance was only slightly reduced by UV-B radiation, but was greatly decreased during the first day of recovery from water

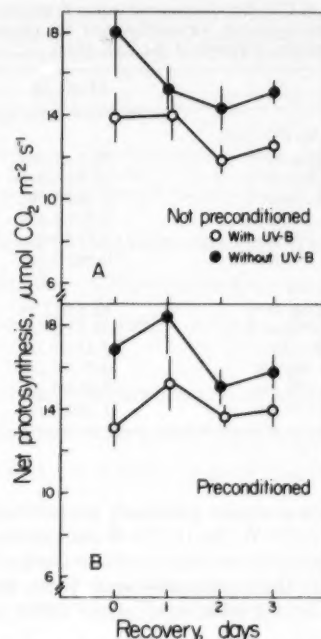


Fig. 3. Photosynthetic recovery from water stress in plants during early vegetative (30-day-old) development. Each symbol represents the mean of 10 measurements and vertical bars their SE. A, change in net photosynthesis in plants not previously preconditioned to water stress; B, change in plants preconditioned to 2 drying cycles to -2.0 MPa leaf ψ .

stress. Dark respiration was also substantially reduced by water stress and did not return to pre-stress levels during the 3 day recovery period.

Tab. 1. The effects of UV-B radiation on diffusive conductance and dark respiration during photosynthetic recovery from water stress in vegetative soybean. Plants were either continuously well-watered prior to water stress (non-preconditioned –UV+W and +UV+W) or previously preconditioned (–UV–W and +UV–W) to 2 drying cycles down to -2.0 MPa leaf ψ . Values represent the means for 10 plants $\pm$ SE.

	Days after water stress							
	Without UV-B				With UV-B			
	0	1	2	3	0	1	2	3
Non-preconditioned								
Non-stomatal conductance (cm s ⁻¹)	0.18 $\pm$ 0.03	0.24 $\pm$ 0.02	0.16 $\pm$ 0.02	0.16 $\pm$ 0.01	0.15 $\pm$ 0.02	0.26 $\pm$ 0.07	0.14 $\pm$ 0.02	0.14 $\pm$ 0.01
Stomatal conductance (cm s ⁻¹)	0.42 $\pm$ 0.05	0.27 $\pm$ 0.04	0.37 $\pm$ 0.03	0.42 $\pm$ 0.02	0.37 $\pm$ 0.05	0.26 $\pm$ 0.04	0.38 $\pm$ 0.04	0.33 $\pm$ 0.03
Dark respiration (μmol CO ₂ m ⁻² s ⁻¹)	3.5 $\pm$ 0.7	1.5 $\pm$ 0.3	1.5 $\pm$ 0.3	1.2 $\pm$ 0.3	3.4 $\pm$ 0.3	2.3 $\pm$ 0.4	1.6 $\pm$ 0.3	1.5 $\pm$ 0.3
Preconditioned								
Non-stomatal conductance (cm s ⁻¹)	0.17 $\pm$ 0.03	0.27 $\pm$ 0.03	0.16 $\pm$ 0.02	0.16 $\pm$ 0.01	0.12 $\pm$ 0.02	0.21 $\pm$ 0.04	0.16 $\pm$ 0.01	0.15 $\pm$ 0.01
Stomatal conductance (cm s ⁻¹)	0.26 $\pm$ 0.01	0.31 $\pm$ 0.05	0.33 $\pm$ 0.09	0.37 $\pm$ 0.04	0.23 $\pm$ 0.05	0.28 $\pm$ 0.03	0.38 $\pm$ 0.04	0.48 $\pm$ 0.02
Dark respiration (μmol CO ₂ m ⁻² s ⁻¹)	3.5 $\pm$ 0.2	1.5 $\pm$ 0.4	2.4 $\pm$ 0.3	0.7 $\pm$ 0.2	2.7 $\pm$ 0.3	1.5 $\pm$ 0.5	1.6 $\pm$ 0.5	1.5 $\pm$ 0.3

Tab. 2. Effects of UV-B radiation and water stress on chlorophyll concentration and methanolic extract absorbance in vegetative and reproductive soybeans. Values represent the means for 20 determinations $\pm$ 1 sd. Values followed by different letters in each row are significantly different at the 95% level.

	+UV+W	-UV+W	+UV-W	-UV-W
Vegetative				
Chlorophyll <i>a</i> (mg m ⁻²)	76.1 $\pm$ 1.1ab	88.6 $\pm$ 7.5a	73.4 $\pm$ 8.4b	82.1 $\pm$ 6.2a
Chlorophyll <i>b</i> (mg m ⁻²)	32.8 $\pm$ 6.8ab	36.7 $\pm$ 3.2a	32.1 $\pm$ 4.3ab	34.2 $\pm$ 2.1a
Chlorophyll <i>a/b</i> ratio	2.33 $\pm$ 0.9bc	2.45 $\pm$ 0.08a	2.29 $\pm$ 0.10c	2.40 $\pm$ 0.11ab
Absorbance at 340 nm	0.76 $\pm$ 0.10a	0.54 $\pm$ 0.13b	0.70 $\pm$ 0.12a	0.55 $\pm$ 0.12b
Absorbance at 290 nm	0.45 $\pm$ 0.06a	0.26 $\pm$ 0.07b	0.40 $\pm$ 0.10a	0.26 $\pm$ 0.12b
Absorbance at 270 nm	0.66 $\pm$ 0.10a	0.32 $\pm$ 0.10b	0.55 $\pm$ 0.17a	0.32 $\pm$ 0.16b
Reproductive				
Chlorophyll <i>a</i> (mg m ⁻²)	82.8 $\pm$ 12.1a	72.2 $\pm$ 10.2b	86.0 $\pm$ 12.7a	91.8 $\pm$ 12.9
Chlorophyll <i>b</i> (mg m ⁻²)	35.5 $\pm$ 8.7a	32.7 $\pm$ 4.9ab	37.3 $\pm$ 7.6a	38.1 $\pm$ 8.1a
Chlorophyll <i>a/b</i> ratio	2.33 $\pm$ 0.17a	2.8 $\pm$ 0.14a	2.30 $\pm$ 0.16a	2.45 $\pm$ 0.49a
Absorbance at 340 nm	0.77 $\pm$ 0.10a	0.58 $\pm$ 0.12b	0.72 $\pm$ 0.17a	0.53 $\pm$ 0.10b
Absorbance at 290 nm	0.88 $\pm$ 0.10a	0.83 $\pm$ 0.13ab	0.79 $\pm$ 0.16b	0.77 $\pm$ 0.08b
Absorbance at 270 nm	1.25 $\pm$ 0.13a	1.08 $\pm$ 0.17ab	1.10 $\pm$ 0.23ab	1.03 $\pm$ 0.10b

In vegetative soybeans previously preconditioned to water stress (+UV-W; Trt 3), UV-B also produced substantial reductions in net photosynthesis compared with control plants. These preconditioned plants, however, appeared to recover from water stress within one day

after rewatering (Fig. 3B). A trend toward gradually increasing stomatal conductance was evident during recovery in both irradiated and non-irradiated plants (Tab. 1). Reduced net photosynthesis in UV-B irradiated compared with non-irradiated plants was significantly correlated ($r = 0.78$, $P < 0.01$) with lower non-stomatal conductance. Dark respiration was also reduced by water stress and did not recover within the 3 day observation period.

During vegetative growth, UV-B radiation resulted in reductions in the amounts of both chlorophyll *a* and *b*, regardless of previous preconditioning to water stress (+UV+W and +UV-W; Tab. 2). Chlorophyll *a/b* ratio was also lower in UV-B irradiated plants than in controls. Methanolic extract absorbance increased in UV-B irradiated plants while water stress had little effect on this parameter.

During early reproductive growth, net photosynthesis in non-preconditioned plants (Trts 1 and 2) was much more sensitive to water stress than in preconditioned plants (Fig. 4A). Even 3 days after rewatering, photosynthetic recovery from water stress was incomplete and rates were still 20–40% below the initial values for well-watered plants. This large, initial reduction in net photosynthesis was accompanied by a combination of trends in decreased stomatal and non-stomatal conductances to CO₂ diffusion into the leaf and a gradual increase in dark respiration (Tab. 3). Although stomatal conductance recovered to pre-stressed levels, non-stomatal conductance remained significantly lower in all treatments except +UV-W (Trt 3).

Net photosynthesis in preconditioned plants was not reduced to the same degree as in non-preconditioned plants (Fig. 4B). Both groups of preconditioned plants experienced the same relative reduction in net photosynthesis the first day after stress; however, UV-B irradiated plants (+UV-W; Trt 3) were fully recovered by day 2, while non-irradiated (-UV-W; Trt 4) remained at

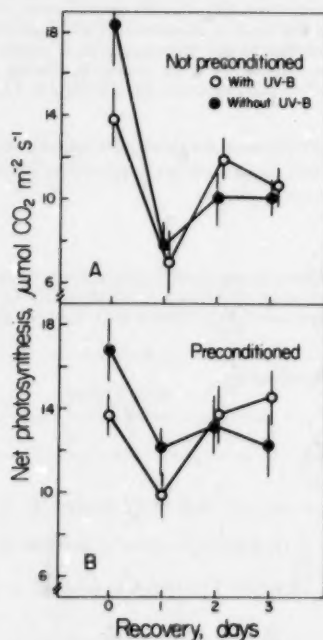


Fig. 4. Photosynthetic recovery from water stress in plants during early reproductive (60-day-old) development. Each symbol represents the mean of 10 measurements and vertical bars their SE. A, change in net photosynthesis in plants not previously preconditioned to water stress; B, change in plants preconditioned to 2 drying cycles to -2.0 MPa leaf ψ .

Tab. 3. The effects of UV-B radiation on diffusive conductance and dark respiration during photosynthetic recovery from water stress in reproductive soybean. Plants were either continuously well-watered prior to water stress (non-preconditioned -UV+W and +UV+W) or previously preconditioned (-UV-W and +UV-W) to 2 drying cycles down to -2.0 MPa leaf ψ . Values represent the means for 10 plants $\pm$ 1 se.

	Days after water stress							
	Without UV-B				With UV-B			
	0	1	2	3	0	1	2	3
<i>Non-preconditioned</i>								
Non-stomatal conductance (cm s ⁻¹)	0.18 $\pm$ 0.03	0.05 $\pm$ 0.02	0.09 $\pm$ 0.02	0.10 $\pm$ 0.02	0.15 $\pm$ 0.02	0.09 $\pm$ 0.04	0.12 $\pm$ 0.02	0.10 $\pm$ 0.01
Stomatal conductance (cm s ⁻¹)	0.42 $\pm$ 0.05	0.22 $\pm$ 0.02	0.26 $\pm$ 0.03	0.48 $\pm$ 0.07	0.37 $\pm$ 0.05	0.19 $\pm$ 0.02	0.26 $\pm$ 0.01	0.42 $\pm$ 0.09
Dark respiration (μ mol CO ₂ m ⁻² s ⁻¹)	3.5 $\pm$ 0.7	3.8 $\pm$ 0.6	3.3 $\pm$ 0.4	4.7 $\pm$ 0.4	3.4 $\pm$ 0.3	3.5 $\pm$ 0.4	3.9 $\pm$ 0.5	4.6 $\pm$ 0.4
<i>Preconditioned</i>								
Non-stomatal conductance (cm s ⁻¹)	0.17 $\pm$ 0.03	0.11 $\pm$ 0.01	0.12 $\pm$ 0.03	0.11 $\pm$ 0.02	0.13 $\pm$ 0.02	0.12 $\pm$ 0.02	0.13 $\pm$ 0.03	0.16 $\pm$ 0.02
Stomatal conductance (cm s ⁻¹)	0.26 $\pm$ 0.04	0.28 $\pm$ 0.01	0.33 $\pm$ 0.02	0.59 $\pm$ 0.10	0.23 $\pm$ 0.05	0.23 $\pm$ 0.05	0.26 $\pm$ 0.03	0.48 $\pm$ 0.09
Dark respiration (μ mol CO ₂ m ⁻² s ⁻¹)	3.2 $\pm$ 0.2	4.6 $\pm$ 0.4	3.8 $\pm$ 0.6	4.9 $\pm$ 0.4	2.7 $\pm$ 0.3	4.8 $\pm$ 0.4	3.6 $\pm$ 0.6	4.5 $\pm$ 0.4

Tab. 4. The effects of UV-B radiation and water stress on several plant and growth characteristics in soybean during vegetative growth (V-3). All plants were 36 days old. Values represent the means for 10 plants $\pm$ 1 sd. Values followed by a different letter in each row are significantly different at the 95% level. DW, dry weight.

Plant characteristic	+UV+W	-UV+W	+UV-W	-UV-W
Plant height (m)	0.56 $\pm$ 0.03a	0.57 $\pm$ 0.03a	0.55 $\pm$ 0.04a	0.56 $\pm$ 0.02a
Number of nodes	10.6 $\pm$ 0.7ab	11.0 $\pm$ 0.5a	10.4 $\pm$ 0.7ab	10.2 $\pm$ 0.4b
Number of leaves	13.2 $\pm$ 1.2a	12.2 $\pm$ 1.1b	10.1 $\pm$ 0.9c	9.7 $\pm$ 1.4c
Leaf area (m ²)	0.11 $\pm$ 0.02a	0.11 $\pm$ 0.01a	0.08 $\pm$ 0.01b	0.08 $\pm$ 0.01b
SLW (g m ⁻² $\times$ 10 ²)	0.27 $\pm$ 0.02a	0.27 $\pm$ 0.01a	0.25 $\pm$ 0.02ab	0.24 $\pm$ 0.01b
Total DW (g)	6.83 $\pm$ 0.79b	8.07 $\pm$ 1.54a	4.82 $\pm$ 1.0c	5.40 $\pm$ 0.79c
% Leaves	42.3 $\pm$ 2.4a	38.6 $\pm$ 4.2bc	41.4 $\pm$ 2.5ab	37.5 $\pm$ 3.6c
% Stems	31.4 $\pm$ 1.7a	26.6 $\pm$ 3.1b	28.3 $\pm$ 2.2b	25.9 $\pm$ 2.1b
% Petioles	1.4 $\pm$ 0.1a	1.3 $\pm$ 0.2ab	1.3 $\pm$ 0.1ab	1.1 $\pm$ 0.1b
% Roots	24.9 $\pm$ 2.9c	33.5 $\pm$ 7.2ab	29.1 $\pm$ 3.7bc	35.5 $\pm$ 5.5a

a constant but lower rate (25-30% below pre-stressed values). This rapid photosynthetic recovery was correlated with a gradual increase in both stomatal and non-stomatal conductance in irradiated plants (Tab. 3). In non-irradiated plants, stomatal conductance also increased, yet the more limiting non-stomatal component remained at a low level.

During early reproductive growth, the amounts of chlorophyll *a* and *b* were lowest in leaves of -UV+W (Trt 2) plants (Tab. 2). Methanolic extract absorbance was generally greater in UV-B irradiated plants; however, water stress was somewhat effective in reducing absorbance. Therefore the greatest absorbance was found in +UV+W (Trt 1) plants while the least was found in -UV-W (Trt 4) plants.

Plant growth

After 36 days growth under a combination of UV-B

radiation and water stress, most of the plant characteristics measured were significantly affected by one or the other of the stresses (Tab. 4). In +UV+W (Trt 1) plants, there was a significant decrease in root:shoot ratios evidenced by an increase in leaf number and the proportion of biomass allocated into aboveground plant parts (leaves, stems, and petioles) and a 30% smaller root biomass. Water stress was effective in reducing the number of plant nodes and leaves, and decreased leaf area and specific leaf weight (SLW), the ratio of leaf mass to area. The combination of UV-B and water stress (Tab. 4) produced an additive adverse effect on productivity (estimated by total dry weight accumulation). The highest productivity was found in -UV+W (Trt 2) plants, while the least was in those plants simultaneously exposed to both stresses (Trt 3).

The effects of UV-B and water stress on soybean growth during reproductive development were generally smaller than observed in vegetative plants (Tab. 5).

Tab. 5. The effects of UV-B radiation and water stress on several plant and growth characteristics during reproductive growth (R-2). All plants were 60 days old. Values are the means for 10 plants $\pm$ 1 sd. Values followed by a different letter in each row are significantly different at the 95% level. DW, dry weight.

Plant characteristic	+UV+W	-UV+W	+UV-W	-UV-W
Plant height (m)	0.96 $\pm$ 0.09a	0.91 $\pm$ 0.09a	0.98 $\pm$ 0.16a	0.93 $\pm$ 0.14a
Number of nodes	13.6 $\pm$ 1.1a	12.4 $\pm$ 0.5a	12.9 $\pm$ 0.9a	12.5 $\pm$ 0.8a
Number of leaves	23.0 $\pm$ 4.8a	24.4 $\pm$ 4.0a	21.7 $\pm$ 5.6a	22.9 $\pm$ 4.3a
Leaf area (m ²)	0.25 $\pm$ 0.03b	0.29 $\pm$ 0.02a	0.27 $\pm$ 0.03ab	0.28 $\pm$ 0.02a
SLW (g m ⁻² $\times$ 10 ²)	0.41 $\pm$ 0.05a	0.33 $\pm$ 0.02b	0.32 $\pm$ 0.05b	0.31 $\pm$ 0.02b
Total DW (g)	24.4 $\pm$ 3.1a	22.2 $\pm$ 2.3ab	19.9 $\pm$ 4.5b	19.8 $\pm$ 2.0b
% Leaves	41.4 $\pm$ 2.9a	43.1 $\pm$ 1.5a	43.8 $\pm$ 2.3a	43.1 $\pm$ 2.7a
% Stems	35.8 $\pm$ 2.4b	38.8 $\pm$ 1.5a	37.7 $\pm$ 1.7a	37.0 $\pm$ 3.3a
% Petioles	0.1 $\pm$ 0b	0.2 $\pm$ 0a	0.1 $\pm$ 0b	0.2 $\pm$ 0a
% Roots	22.6 $\pm$ 3.8a	17.9 $\pm$ 1.5b	18.3 $\pm$ 2.5b	19.0 $\pm$ 2.9b

Water stress resulted in a small decrease in total dry weight, but the combination of UV-B and water stress produced no further reductions in biomass.

Discussion

During early stages of vegetative growth, both UV-B and water stress reduced total plant dry weight. Compared with water stress, UV-B was more effective in changing biomass allocation into various organs. Water stress, on the other hand, produces greater reductions in leaf area and leaf and node number. The combination of UV-B and water stress produced an additive effect on total dry matter production.

This combined, adverse effect on productivity was also evident in the effects of UV-B and water stress on net photosynthesis. Regardless of any previous preconditioning to water stress, UV-B irradiated leaves always had lower absolute rates of net photosynthesis. Water stress resulted in an additional reduction in this already depressed rate. UV-B-induced reductions in net photosynthesis have been previously reported for soybean (Teramura et al. 1980, Vu et al. 1982) as well as for other crops (Teramura 1983) and native species (e.g. Caldwell et al. 1982). Reductions in net photosynthesis occurred despite a 30–40% UV-B-induced increase in flavonoids (methanolic extracts). The production of flavonoids is thought to be an adaptive response which protects sensitive organelles from the deleterious effects of UV-B radiation (Wellmann 1982). Several other studies have also shown that UV-B reduced net photosynthesis despite a substantial increase in methanolic extract absorbance (Sisson 1981, Mirecki and Teramura 1984). Under high UV-B irradiance, flavonoid biosynthesis is eventually inhibited in some plants such as bean and cucumber (Tevini et al. 1983a). Therefore, the effectiveness of flavonoids as a protective mechanism seems to be both species and UV-B dose dependent.

Analysis of CO₂ and H₂O vapor fluxes has revealed that UV-B primarily limits net photosynthesis by decreasing non-stomatal (mesophyll) conductance

(Teramura et al. 1980, Brandle et al. 1977), although a few studies have shown that UV-B can also affect stomatal conductance in sensitive plants (Teramura et al. 1983, Sisson and Caldwell 1976). The reduction in non-stomatal conductance may arise from the deleterious effect of UV-B upon photosystem II as evidenced by reductions in Hill activity (Brandle et al. 1977, Vu et al. 1981) and in variable fluorescence (Noorudeen and Kulandaivelu 1982, Iwanzik et al. 1983).

On an absolute basis, UV-B had little effect on the photosynthetic recovery from water stress in vegetative soybean plants. In contrast, preconditioning to water stress largely determined the extent of photosynthetic recovery. Plants not previously preconditioned to water stress did not photosynthetically recover within a 3 day period from a single exposure to a leaf ψ of -2.0 MPa.

In other studies, dark respiration has been shown to be affected after only 6 h of UV-B irradiation in *Pisum sativum* (Brandle et al. 1977) and after several days in *Rumex patientia* (Sisson and Caldwell 1976). However, the UV-B irradiance supplied in those studies was equivalent to much greater (38–50%) ozone depletions than used in this study (23%). Dark respiration was unaffected by UV-B in the present study, in agreement with previous reports on soybean (Teramura et al. 1980, Mirecki and Teramura 1984).

Growth in 60-day-old, reproductive plants was not as sensitive to the combination of UV-B and water stress as were vegetative plants. In fact, plants exposed to the combination of stresses simultaneously did not differ substantially from the well-watered, non-irradiated (control) plants. This was partially anticipated, since plants at this stage of development had produced most of their biomass in the absence of water stress. The reduced UV-B sensitivity in growth characteristics found in older soybean plants may also be related to the increased level of flavonoids produced in the leaves of irradiated plants. Concentrations of flavonoids increased between 3- to 5-fold in 60-day-old plants compared with those of 36-day-old plants.

Although the treatment combination produced only

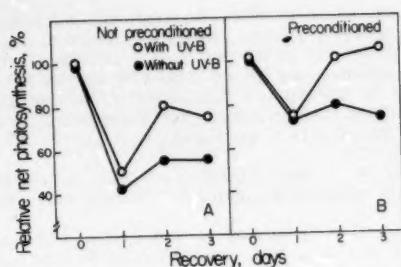


Fig. 5. Relative photosynthetic recovery from water stress in 60-day-old plants, expressed as a percentage of pre-stressed values. A, not preconditioned to water stress; B, previously preconditioned to 2 drying cycles to -2.0 MPa leaf ψ .

small effects on overall plant growth in early reproductive soybeans, a ψ of -2.0 MPa substantially limited plant gas exchange. Plants previously preconditioned to water stress were less severely affected and recovered to a greater extent than continuously well-watered plants. On an absolute basis, UV-B had only relatively small effects on photosynthetic recovery. However, when expressed on a relative basis, UV-B irradiated leaves recovered from water stress much more rapidly and to a greater extent (Fig. 5). This greater photosynthetic recovery (on a relative basis) in UV-B irradiated leaves was coincident with higher, and relatively less water stress-sensitive non-stomatal conductance. The mechanism for such UV-B effects on the photosynthetic recovery from water stress in soybean is presently unknown. However, it seems that UV-B and water stress inhibit photosynthesis by different mechanisms. Kaiser et al. (1981) have shown that water stress apparently does not directly affect the light reactions of photosynthesis, but rather has an indirect effect on inhibiting soluble enzymes in the chloroplast stroma, due to the increase in internal solute concentration caused by the reduction in cell and organelle volumes. On the other hand, studies by Noorudeen and Kulandaivelu (1982) and Iwanzik et al. (1983) suggest that UV-B has a direct effect on the PS II reaction center.

The greater sensitivity of young, vegetative plants to UV-B radiation was not only apparent from the reductions in plant biomass and net photosynthesis, but also from an analysis of chlorophyll. Total chlorophyll and chlorophyll *a/b* ratios were reduced in irradiated plants. Similar reductions after seedlings were UV-B irradiated have been reported by Tevini et al. (1983a) and Vu et al. (1981, 1982). In contrast, total chlorophyll was increased by UV-B in reproductive plants. Since reproductive plants with the lowest chlorophyll concentration also maintained the highest rates of photosynthesis (controls), it can be assumed that UV-B induced reductions in photosynthesis were not caused by decreases in chlorophyll in these plants.

In conclusion, the combination of UV-B radiation and

water stress produced a greater reduction in soybean photosynthesis during early vegetative growth than produced by either stress independently. Older, reproductive plants are less sensitive to UV-B than water stress, possibly due to a greater accumulation of flavonoids in irradiated leaves. It would be unlikely for plants in the field to reach reproductive maturity without being subjected to some degree of water stress, and young seedlings in the field could almost certainly experience water stresses similar to those simulated in this study. Under natural conditions then, we might expect plants to be more adversely affected by enhanced UV-B irradiance during the early stages of seedling growth and development. Concomitant periods of water stress would have an additive, deleterious effect on these plants. Work is in progress to determine whether adverse physiological responses are reflected in reductions of total yield and quality of agricultural products.

Acknowledgements – The authors would like to thank Karen Teramura for illustrating all the figures and Drs Irv Forseth, N. S. Murali and Jim Syvertsen for reading an earlier version of this manuscript. Also many thanks to Inez Miller and Tammy Renoud for their careful typing. This work was supported by the United States Environmental Protection Agency's Environmental Research Laboratory in Corvallis, Oregon (CR 808-035-020) and grants from the Graduate School and Provost to the senior author. Scientific Article No. A-3524, Contribution No. 6598, of the Maryland Agricultural Experiment Station, Department of Botany.

Although the work described in this article has been funded in part by the United States Environmental Protection Agency, it has not been subjected to the Agency's required peer and policy review and therefore does not necessarily reflect the views of the Agency and no official endorsement should be inferred.

References

- Arnon, D. I. 1949. Copper enzymes in isolated chloroplasts. Polyphenol oxidase in *Beta vulgaris*. – *Plant Physiol.* 24: 1–15.
- Brandle, J. R., Campbell, W. F., Sisson, W. B. & Caldwell, M. M. 1977. Net photosynthesis, electron transport capacity, and ultrastructure of *Pisum sativum* L. exposed to ultraviolet-B radiation. – *Plant Physiol.* 60: 165–169.
- Caldwell, M. M. 1968. Solar ultraviolet radiation as an ecological factor for alpine plants. – *Ecol. Monogr.* 38: 243–267.
- 1971. Solar UV irradiation and the growth and development of higher plants. – In *Photophysiology* (A. C. Giese, ed.), Vol. 6, pp. 131–177. Academic Press, N.Y.
- 1981. Plant response to solar ultraviolet radiation. – In *Encyclopedia of Plant Physiology*, New Series, Vol. 12A, *Physiological Plant Ecology I* (O. L. Lange, P. S. Nobel, C. B. Osmond, and H. Ziegler, eds), pp. 169–197. Springer-Verlag, Berlin. ISBN 0-387-10763-0.
- , Robberecht, R., Nowak, R. S. & Billings, W. D. 1982. Differential photosynthetic inhibition by ultraviolet radiation in species from the Arctic-Alpine life zone. – *Arctic Alpine Res.* 14: 195–202.
- Fehr, W. R. & Caviness, C. E. 1977. Stages of soybean development. – Cooperative Extension Service, Agriculture and Home Economics Experiment Station, Iowa State U. of Science and Technology. pp. 1–11.

- Goodnight, J. H. 1979. GLM Procedure Reference. - In SAS Users Guide (J. T. Helwig and K. A. Council, eds), pp. 245-264, SAS Institute, Inc., Cary, N.C.
- Hoagland, D. R. & Arnon, D. I. 1950. The water culture method for growing plants. - California Agricultural Experiment Station. Circ. No. 347 pp. 29-32.
- Hsiao, T. C. 1973. Plant responses to water stress. - Annu. Rev. Plant Physiol. 24: 519-570.
- Iwanzik, W., Tevini, M., Dohnt, G., Voss, G., Weiss, W., Gräber, P. & Renger, G. 1983. Action of UV-B radiation on photosynthetic primary reactions in spinach chloroplasts. - Physiol. Plant. 58: 401-407.
- Jarvis, P. G. 1971. The estimation of resistances to carbon dioxide transfer. - In Plant Photosynthetic Production Manual of Methods (Z. Šesták, J. Čatský, and P. G. Jarvis, eds), pp. 566-631, Dr. W. Junk N. V. Publishers, The Hague.
- Kaiser, W. M., Kaiser, G., Schöner, S. & Neimanis, S. 1981. Photosynthesis under osmotic stress: Differential recovery of photosynthetic activities of stroma enzymes, intact chloroplasts, protoplasts, and leaf slices after exposure to high solute concentrations. - Planta 153: 430-435.
- Mirecki, R. M. & Teramura, A. H. 1984. Effects of ultraviolet-B irradiance on soybean. V. The dependence of plant sensitivity on the photosynthetic photon flux density during and after leaf expansion. - Plant Physiol. 74: 1-6.
- National Academy of Sciences. 1982. Causes and Effects of Stratospheric Ozone Reduction: An Update. - National Academy Press, Washington D.C., p. 339. ISBN 0-309-03248-2.
- Noorudeen, A. M. & Kulandaivelu, G. 1982. On the possible site of inhibition of photosynthetic electron transport by ultraviolet-B (UV-B) radiation. - Physiol. Plant. 55: 161-166.
- Salisbury, F. B. & Ross, C. W. 1978. Plant Physiology (2nd Ed.). - Wadsworth Publishing Co., Inc., Belmont, Ca. pp. 361-374. ISBN 0-534-00562-4.
- Setlow, R. B. 1974. The wavelengths in sunlight effective in producing skin cancer: A theoretical analysis. - Proc. Natl. Acad. Sci. 71: 3363-3366.
- Sisson, W. B. 1981. Photosynthesis, growth and ultraviolet irradiance absorbance of *Cucurbita pepo* L. leaves exposed to ultraviolet-B radiation (280-315 nm). - Plant Physiol. 67: 120-124.
- & Caldwell, M. M. 1976. Photosynthesis, dark respiration, and growth of *Rumex patientia* L. exposed to ultraviolet irradiance (288 to 315 nanometers) simulating a reduced atmospheric ozone column. - Plant Physiol. 58: 563-568.
- Sokal, R. R. & Rohlf, F. J. 1981. Biometry: The Principles and Practice of Statistics in Biological Research (2nd Ed.). - W. H. Freeman and Co., San Francisco, Ca., pp. 321-371. ISBN 0-7167-1254-7.
- Teramura, A. H. 1983. Effects of ultraviolet-B radiation the growth and yield of crop plants. - Physiol. Plant. 58: 415-427.
- , Biggs, R. H. & Kossuth, S. 1980. Effects of ultraviolet-B irradiances on soybean. II. Interaction between ultraviolet-B and photosynthetically active radiation on net photosynthesis, dark respiration, and transpiration. - Plant Physiol. 65: 483-488.
- , Tevini, M. & Iwanzik, W. 1983. Effects of ultraviolet-B irradiation on plants during mild water stress. I. Effects on diurnal stomatal resistance. - Physiol. Plant. 57: 175-180.
- Tevini, M., Thoma, U. & Iwanzik, W. 1983a. Effects of enhanced UV-B radiation on germination, seedling growth, leaf anatomy and pigments of some crop plants. - Z. Pflanzenphysiol. 109: 435-448.
- , Iwanzik, W. & Teramura, A. H. 1983b. Effects of UV-B irradiation on plants during mild water stress. II. Effects on growth, proteins, and flavonoids. - Z. Pflanzenphysiol. 110: 459-467.
- Vu, C. V., Allen, Jr., L. H. & Garrard, L. A. 1981. Effects of supplemental UV-B radiation on growth and leaf photosynthetic reactions of soybean (*Glycine max*). - Physiol. Plant. 52: 353-362.
- , Allen, Jr., L. H. & Garrard, L. A. 1982. Effects of UV-B radiation (280-320 nm) on photosynthetic constituents and processes in expanding leaves of soybean [*Glycine max* (L.) Merr.]. - Environ. Exp. Bot. 22: 465-473.
- Wellmann, E. 1982. Phenylpropanoid pigment synthesis and growth reduction as adaptive reactions to increased UV-B radiation. - In Biological Effects of UV-B Radiation (H. Bauer, M. M. Caldwell, M. Tevini and R. C. Worrest, eds), pp. 145-149, Gesellschaft für Strahlen- und Umweltforschung mbH, München.